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Synthesis and Pharmacological Activities of 6-Glycine Substituted 14-Phenylpropoxymorphinans, a Novel Class of Opioids with High Opioid Receptor Affinities and Antinociceptive Potencies[†]

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The synthesis and the effect of a combination of 6-glycine and 14-phenylpropoxy substitutions in *N*-methyl- and *N*-cycloproplymethylmorphinans on biological activities are described. Binding studies revealed that all new 14-phenylpropoxymorphinans (**11**−**18**) displayed high affinity to opioid receptors. Replacement of the 14-methoxy group with a phenylpropoxy group led to an enhancement in affinity to all three opioid receptor types, with most pronounced increases in δ and κ activities, hence resulting in a loss of μ receptor selectivity. All compounds (**11**−**18**) showed potent and long-lasting antinociceptive effects in the tail-flick test in rats after subcutaneous administration. For the *N*-methyl derivatives **13** and **14**, analgesic potencies were in the range of their 14-methoxy analogues **9** and **10**, respectively. Even derivatives **15**−**18** with an *N*-cyclopropylmethyl substituent acted as potent antinociceptive agents, being several fold more potent than morphine. Subcutaneous administration of compounds **13** and **14** produced significant and prolonged antinociceptive effects mediated through peripheral opioid mechanisms in carrageenan-induced inflammatory hyperalgesia in rats.

Introduction

The clinical management of pain, especially severe and chronic pain, is still a major challenge.[1,2] Analgesic drugs such as opioids play a central role in pain control.[2,3] Together with endogenous opioids, they modulate nociceptive transmission at different levels in the pain modulating pathways via interaction with opioid receptors.[4−6] The efficacy of currently used opioid analgesics, including morphine, oxymorphone, oxycodone, and fentanyl, is frequently associated with the occurrence of undesired dose-limiting side effects.[2,3] There is a continued search for opioids that are highly efficacious with reduced complications and improved patient compliance.

Work in our laboratory has been focused on the development of new opioid agonists and antagonists from the morphinan class of compounds.[7−9] Introduction of a 14-methoxy group in oxymorphone resulted in 14-*O*-methyloxymorphone[10] (**1**; Figure 1), which not only increases affinity to opioid receptors while retaining the μ receptor selectivity but also markedly enhances the antinociceptive potency.[10−12] Further work on 14-alkoxymorphinans led to the development of 14-methoxymetopon[13] (**2**; Figure 1). It was reported as a selective and high efficacy μ opioid receptor agonist showing potent centrally mediated antinociceptive effects and less pronounced typical opioid adverse actions.[12−18] A derivative of the 14-alkoxymorphinan series of opioids, 14-phenylpropoxymetopon[19] (**3**; Figure 1), has been described as an extremely powerful analgesic with high affinity for all three opioid receptor types (μ, δ, and κ).[19] Similar results were provided on differently *N*-substituted 14-phenylpropoxymorphinan-6-ones (e.g., *N*-allyl and *N*-cyclopropylmethyl substituted morphinans **5** and **6**).[20] It was established that the presence of a 14-phenylpropoxy group increases both the agonist potency and the affinity for all three opioid receptor types while concurrently diminishing the selectivity for any of the receptors. The two classical opioid antagonists naloxone (**7**) and naltrexone (**8**) were converted into highly active analgesic agents by introducing a phenylpropoxy group in position 14 (compounds **5** and **6**, respectively).[20] Moreover, derivatives of the selective μ opioid receptor antagonist cyprodime having a phenylpropoxy group at C-14 have also acted as potent antinociceptives in different pain models in mice after subcutaneous (sc) administration.[21] Thus, the presence of this substituent directs to a profound alteration in the pharmacological profile of morphinan-6-ones.

[†]This paper is dedicated to Dr. Kenner C. Rice on the occasion of his 70th birthday.

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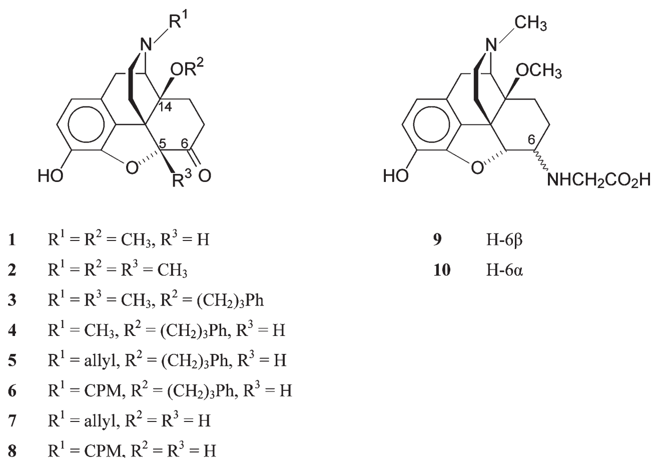


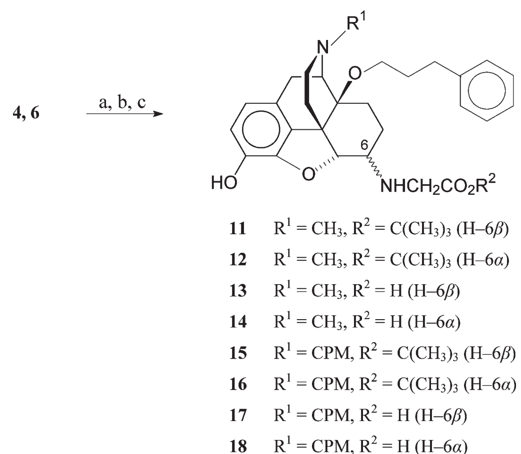
Figure 1. Structures of ligands related to 14-*O*-methyloxymorphone (**1**), naloxone (**7**), and naltrexone (**8**). CPM, cycloproplymethyl; Ph, phenyl.

Traditionally, analgesic effects of opioids have been associated with exclusive activation of opioid receptors in the central nervous system (CNS[a]). There is large evidence that intrinsic pain control can also occur at peripheral sites,[6,22−24] which is supported by the identification of peripheral opioid receptors on sensory neurons.[25,26] The contribution of the opioid system to peripheral pain control mechanisms gained considerable attention during the past years, leading to new directions in research focusing on exploration of the therapeutic potential of peripheral opioid receptors for superior management of pain.[6,23,24,27,28] Targeting peripheral mechanisms can provide selective peripheral analgesia by avoiding the central complications associated with the use of opioids.[6,23,27]

Our research in the field of peripherally acting opioid antinociceptive agents has led us to obtain a series of 6-amino acid substituted derivatives (glycine, alanine, and phenylalanine) of 14-*O*-methyloxymorphone (**1**).[29] These compounds displayed high affinities at the μ opioid receptor and showed potent agonism.[30] A number of pharmacological studies reported that amino acid substitution in position 6 of 14-*O*-methyloxymorphone affords derivatives that produce potent antinociceptive actions in rodent models of acute nociception, inflammatory, visceral, and neuropathic pain.[18,31−33] These antinociceptive effects were shown to be mediated by activating primarily peripheral opioid receptors. They showed markedly long-lasting antinociceptive actions compared to the conventional centrally acting μ opioids, fentanyl, morphine, the parent compound **1**, and 14-methoxymetopon (**2**).[8,31−33] The most potent compounds were the 6-glycine, 14-methoxy substituted derivatives **9** and **10**, respectively (Figure 1). The 6β-glycine analogue **10** exhibited an antinociceptive potency comparable to fentanyl and was only 2-fold lower than that of 14-*O*-methyloxymorphone (**1**) after sc administration.[31]

In the present study, we have investigated the effect of a combination of 6-amino acid and 14-phenylpropoxy substitutions in *N*-methyl- and *N*-cycloproplymethylmorphinans on the biological profile represented by in vitro binding and antinociceptive activities after sc administration to rats. Structure−activity relationship (SAR) studies relating to the substitution pattern in positions 6 and 14 within this series were pursued. To this aim, 6-glycine derivatives having a 14-phenylpropoxy group (compounds **13**, **14**, **17**, and **18**) were synthesized and their in vitro and in vivo opioid activities were evaluated. We have also assessed the peripheral mechanisms of antinociceptive effects of derivative **13** and **14** after sc administration to rats with carrageenan-induced inflammatory pain. In addition, the pharmacological properties of the corresponding *tert*-butyl esters **11**, **12**, **15**, and **16** were investigated in order to extend the SAR in the series of 14-alkoxymorphinans.

Scheme 1. Synthesis of 14-Phenylpropxymorphinans **11−18**[a]



[a] Reagents and conditions: (a) glycine-*t*-butylester hydrochloride, $NaCNBH_3$, DMF/MeOH 10:1, RT; (b) separation of the diastereoisomers using column chromatography (silica gel); (c) 4 M HCl in dioxane, reflux.

Chemistry. Reductive amination of the 14-phenylpropoxymorphinan-6-ones **4**[21] and **6**[20] was performed with glycine *tert*-butyl ester hydrochloride and $NaCNBH_3$ in DMF/MeOH 10:1 at room temperature (Scheme 1). The diastereoisomers were separated by medium pressure liquid chromatography (MPLC) to obtain **11**, **12**, **15**, and **16**. Ester cleavage of the *tert*-butyl derivatives in dioxane/HCl generated the amino acids **13**, **14**, **17**, and **18**. Configuration assignments at C(6) are based on the coupling constants (J(5,6)) between H−C(5) and H−C(6). J(5,6) values for 6α-amino epimers are smaller (3.2−4.0 Hz) than for 6β-amino epimers (6.5−7.8 Hz).[29,34,35] The results for compounds **11−18** agree with the earlier findings.

Results and Discussion

In Vitro Opioid Receptor Binding Affinities. Binding affinities of the newly synthesized compounds **11−18** at opioid receptors were determined by in vitro competition binding assays using rat brain (μ, δ) and guinea pig brain (κ) membranes and employing [^{3}H][D-Ala^2,Me-Phe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO, μ),[36] [^{3}H][Ile^{5,6}]deltorphin II (δ),[37] and [^{3}H]-(5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4−5)dec-8-yl]benzeneacetamide) ([^{3}H]U69,593, κ)[38] as specific opioid radioligands.[30] The μ, δ, and κ opioid receptor binding affinities expressed as inhibition constants (K_i) are summarized in Table 1. The selectivity for the μ opioid receptor vs δ and κ receptors was defined by the ratio of

[a] Abbreviations: CHO, Chinese hamster ovary; CNS, central nervous system; DAMGO, [D-Ala^2,Me-Phe^4,Gly-ol^5]enkephalin; DMF, *N*,*N*-dimethylformamide; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GDP, guanosine diphosphate; [^{35}S]GTPγS, guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate; MPLC, medium pressure liquid chromatography; SAR, structure−activity relationship; Tris, tris-(hydroxymethyl)-aminomethane; U69,593, 5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4−5)dec-8-yl]-benzeneacetamide.

Table 1. Binding Affinities at μ, δ and κ Opioid Receptors

	K_i (nM)[a]			selectivity ratio	
compd	μ receptor [^{3}H]DAMGO[b]	δ receptor [^{3}H][Ile^{5,6}]deltorphin II[b]	κ receptor [^{3}H]U69,593[c]	δ/μ	κ/μ
morphine[d]	6.55 ± 0.74	217 ± 19	113 ± 9[a]	33	17
3[e]	0.20 ± 0.05	0.14 ± 0.02	0.54 ± 0.15	0.7	2.7
6[f]	0.34 ± 0.06	0.48 ± 0.05	0.41 ± 0.09	1.4	1.2
9[g]	0.89 ± 0.09	15.4 ± 1.4	43.2 ± 7.0[a]	17	49
10[g]	0.83 ± 0.02	7.86 ± 0.64	44.8 ± 0.1[a]	10	54
11	1.40 ± 0.07	1.01 ± 0.06	1.26 ± 0.30	0.7	0.9
12	1.03 ± 0.25	0.92 ± 0.16	2.02 ± 0.68	0.9	2.0
13	0.19 ± 0.02	0.22 ± 0.02	0.73 ± 0.01	1.2	3.8
14	0.16 ± 0.02	0.19 ± 0.01	0.81 ± 0.03	1.2	5.1
15	1.38 ± 0.21	1.11 ± 0.07	1.49 ± 0.27	0.8	1.1
16	1.01 ± 0.21	0.40 ± 0.07	1.77 ± 0.37	0.4	1.8
17	0.27 ± 0.02	0.33 ± 0.10	0.64 ± 0.01	1.2	2.4
18	0.20 ± 0.04	0.35 ± 0.04	0.65 ± 0.01	1.7	3.2

[a] Values represent the mean ± SEM. [b] Rat brain membranes were used. [c] Guinea pig brain membranes were used. [d] From ref 17. [e] From ref 19. [f] From ref 20. [g] From ref 30.

the K_i values. For comparison purposes, the opioid binding affinity data for morphine, **3**, the 6α- and 6β-glycine conjugates of 14-*O*-methyloxymorphone, **9** and **10**, respectively, and the 14-phenylpropoxy analogue of naltrexone, compound **6**, are included. All compounds **11**−**18** bound with high affinity at the μ opioid receptor (K_i = 0.16−1.40 nM), having considerably improved interaction with the μ site compared to morphine. As shown in Table 1, they also exhibit increased binding affinities at δ and κ receptors as indicated by the K_i values in the subnanomolar or low nanomolar range.

To investigate the SAR for the novel 14-alkoxymorphinans, the following structural modifications were targeted: (i) replacement of the methoxy group with a phenylpropoxy group in position 14, (ii) substitution of the *N*-methyl with an *N*-cyclopropylmethyl group, (iii) modification of the substituent at position 6 (e.g., 6-glycine vs 6-glycine ester vs 6-keto), and (iv) α vs β orientation of the amino acid residue at C-6 of the morphinan skeleton. First, we have examined the result of the replacement of the 14-methoxy group in *N*-methyl substituted morphinans **9** and **10** with a phenylpropoxy group, leading to compounds **13** and **14**, respectively, on the in vitro opioid binding profile. While the μ affinity was less affected, affinities at δ and κ receptors were increased significantly after introduction of a phenylpropoxy substituent (Table 1). Compared to analogues **9** and **10**, affinities of the 14-phenylpropoxy substituted derivatives **13** and **14** were increased by about 7- and 5-fold at μ, 70- and 42-fold at δ, and 59- and 55-fold at κ receptors, respectively. On the basis of these data, it appears that a phenylpropoxy group in position 14 is not favorable for selective binding to μ, δ, or κ receptors by causing a notable loss in μ receptor selectivity, thus, corroborating and extending our previous findings in other series of 14-phenylpropoxy substituted morphinans.[19,20] The present observations together with earlier reports[10,11,19,20,39,40] provide additional evidence that interaction with opioid receptors is sensitive to the character and length of the substituent in position 14. It has been shown that different substitution patterns at C-14 in morphinan-6-ones give rise to compounds with improved or decreased selectivity for the μ receptor. An enhancement in the binding affinity at δ and κ receptors has been reported with other substituents at position 14 such as benzyloxy or naphthylmethoxy.[11] We have described that a 14-methoxy group results in higher selectivity for the μ receptor than 14-benzyloxy or 14-naphthylmethoxy substitution, indicating that small alkoxy groups are superior over arylalkoxy groups in this respect.[11] In contrast, replacement of the 14-methoxy with a phenylpropoxy group in cyprodime and other 4,5-oxygen bridge opened morphinan-6-ones was found to markedly increase binding affinities at μ but also at δ and κ receptors, however retaining μ selectivity.[21]

The effect on the opioid receptor binding profile upon different substitution at the nitrogen of the morphinan skeleton was also investigated. Replacement of the *N*-methyl group in **13** and **14** by a cyclopropylmethyl group resulted in analogues **17** and **18**, respectively. The *N*-cyclopropylmethyl substitution was well-tolerated as indicated by the high affinity for all μ, δ, and κ receptors (K_i values in the subnanomolar range; Table 1). Further evidence for this observation comes from our previous works where nearly identical affinities for all opioid receptor types were noted for **3**[19] and naltrexone derivative **6**.[20] It appears that modification of the *N*-substituent, methyl vs cyclopropylmethyl, causes no major alterations in binding affinity and selectivity in this series of opioid compounds. In other series of morphinans which are unsubstituted or have a hydroxyl or a small alkoxy group at C-14, substitution of the *N*-methyl by a *N*-cyclopropylmethyl group leads to an increase in affinity at δ and κ opioid receptors but also to a raise in μ affinity, resulting in reduced μ receptor selectivity.[11,41,42] A representative example is the comparison of binding affinities of oxymorphone (μ:δ:κ = 0.97:80.5:61.6)[11] with its *N*-cyclopropylmethyl analogue naltrexone (μ:δ:κ = 0.20:8.70:0.40).[42] On the other hand, it has been shown that replacement of the *N*-methyl group in 14-methoxymetopon (**2**) with a 2-phenylethyl group left the binding affinities at μ and δ opioid receptors essentially unchanged but affected interaction with the κ receptor.[11] In view of these results, the nature of the substituent at the nitrogen has a significant effect on both opioid receptor binding affinity and selectivity of the morphinan class of opioid compounds, depending on the character and length of the substituent in position 14.

The effect on opioid receptor binding affinities upon substitution of a keto group with a glycine residue in position 6 in the *N*-methyl and *N*-cyclopropylmethyl 14-phenylpropoxy substituted morphinans was examined. The 6-glycine conjugates **13** and **14** displayed similar binding affinities to all three opioid receptors as the 6-keto derivative **3**. Moreover, in the *N*-cyclopropylmethyl series, in a direct comparison of

Table 2. Antinociceptive Potencies in the Tail-Flick Test in the Rat after sc Administration

	ED_{50} (nmol/kg, sc), 95% CL[a] time after drug administration				
compd	0.5 h	1 h	2 h	3 h	relative potency[b] morphine = 1
morphine	6053[c] (4037−9080)	7626 (5084−11439)	13285 (8856−19929)		1
9[d]	137 (85.7−219)	58.5[c] (36.5−93.7)	143 (86.8−237)	437 (243−786)	103
10[d]	50.8 (32.7−78.7)	29.0[c] (18.8−44.9)	43.3 (27.9−67.1)		209
11	63.5 (15.4−262)	58.3[c] (18.0−188)	84.0 (31.4−255)	148 (28.4−775)	104
12	164 (78.6−341)	92.3[c] (39.5−215)	124 (53.0−292)	189 (69.8−514)	66
13	127 (65.0−246)	81.1[c] (46.4−139)	87.9 (43.9−176)	116 (48.5−278)	75
14	180 (122−247)	143 (85.8−238)	130[c] (73.4−231)	192 (90.0−414)	47
15	365 (195−687)	348[c] (212−570)	363 (215−615)	427 (205−692)	17
16	33.8 (18.8−60.5)	26.8[c] (14.1−51.0)	34.4 (17.7−66.8)	56.0 (21.9−143)	226
17	1388 (117−16526)	349[c] (113−1072)	433 (172−1084)	543 (180−1636)	17
18	1561 (199−12223)	183 (17.7−1877)	147[c] (13.9−1554)	−[e]	41

[a] Values in paranthesis are 95% confidence limits. [b] Relative potencies were calculated at the peak of action. [c] Peak of effect. [d] From ref 31. [e] −: no dose−response relationship.

the 14-phenylpropoxy analogue of naltrexone (**6**) with the 6-glycine derivatives **17** and **18**, all compounds showed comparable affinities at μ, δ, and κ receptors (Table 1). According to the present results, the 6-glycine derivatives having a 14-phenylpropoxy group maintained the high affinity at the μ opioid receptor of the nonzwitterionic compounds **3** and **6**, which is in agreement with a further SAR report in other series of 14-methoxymorphinans containing zwitterionic moieties such as amino acid residues.[29,30] Thus, it is evident that replacement of the 6-keto group with a glycine residue does not have a detrimental effect on affinity at the μ receptor. Analogous observations were made for β-oxymorphamine[43] and β-naltrexamine[44] when having ionizable moieties at C-6. Also, it has been shown in 4,5-oxygen bridge opened *N*-methylmorphinans that a 6-cyano substituent in comparison to a 6-keto group has a minor impact on the ability to interact with opioid receptors.[45] These findings indicate that replacement of the 6-keto group with other substituents such as amino acids or a cyano group produces only minor changes in the opioid binding profile of morphinans.

The consequence of esterification of the glycine residue at C-6 on binding affinity and selectivity was also investigated. As shown in Table 1, the *tert*-butyl ester derivatives **11**, **12**, **15**, and **16** exhibited a decrease in the affinity at μ, δ, and κ opioid receptors compared to the 6-glycine analogues **13**, **14**, **17**, and **18**, respectively. An exception was ester **16**, which showed comparable δ receptor affinity to analogue **18** (0.40 vs 0.35 nM). However, all esters still display high interaction with opioid receptors as indicated by the K_i values in the low nanomolar range (1.01−1.40 nM at μ, 0.40−1.11 nM at δ, and 1.26−2.02 nM at κ receptors). On the other hand, the esters also showed to lack selectivity to a receptor type similar to the corresponding 6-glycine analogues.

When examining the impact of α/β orientation of the amino acid residue at position 6 on the in vitro opioid activity, it was noted that binding affinities and selectivities of α vs β epimers did not vary greatly in this series of newly synthesized 14-phenylpropoxymorphinans (Table 1).

Pharmacological Activities. The 6-glycine substituted 14-phenylpropoxymorphinans **11**−**18** were further evaluated in vivo for antinociceptive effects in rats after sc administration using the tail-flick test.[31] Their antinociceptive properties were compared to those of morphine and derivatives **9** and **10**. The antinociceptive ED_{50} values are listed in Table 2. All compounds acted as potent antinociceptive agents with long-lasting action when administered sc, with peak ED_{50}s of 26.8−349 nmol/kg. Peak antinociception occurred generally one hour after drug sc administration, and only derivatives **14** and **18** had a peak of action at two hours postinjection. Compared to morphine, the 14-phenylpropoxymorphinans of this series showed 17- to 226-fold increased analgesic potency, with compound **16** displaying the highest potency (Table 2). Antinociceptive potencies of the 6-glycine substituted 14-phenylpropoxy derivatives **13** and **14** were in the range of their 14-methoxy analogues **9** and **10**, respectively (Table 2). It appears that a 14-phenylpropoxy substitution maintains the high analgesic activity, while it largely affects opioid binding affinities and μ receptor selectivity (Table 1).

Traditional and generally accepted SAR models have assigned critical importance in defining the pharmacological profile of agonist/antagonist action of morphinan-6-ones to the substitutent at the morphinan nitrogen.[46−48] Substituents such as cyclopropylmethyl or allyl at the nitrogen have been commonly associated with an antagonist character in this series of opioids. On the other hand, there are a number of examples where substitution at C-14 affords potent analgesics independent of the substituent nature at the morphinan nitrogen.[11,19−21,39,40] Even with a *N*-substituent that is linked with distinct antagonist character such as cyclopropylmethyl, antinociceptive agents can be obtained and examples

Table 3. Stimulation of [^{35}S]GTPγS Binding[a]

	μ receptor		δ receptor		κ receptor	
compd	EC_{50} (nM)	% stim[b]	EC_{50} (nM)	% stim[c]	EC_{50} (nM)	% stim[d]
17	0.69 ± 0.10	34.7 ± 6.6	0.69 ± 0.29	26.1 ± 3.0	1.28 ± 0.35	28.2 ± 1.0
18	1.06 ± 0.28	24.2 ± 3.0	4.24 ± 0.47	34.7 ± 0.6	11.0 ± 1.4	30.4 ± 3.3
morphine	15.6 ± 0.5	93 ± 2.8	316 ± 5	103 ± 7	484 ± 213	62 ± 7

[a] Membranes from CHO cells stably transfected with human μ, δ, or κ opioid receptors were incubated with varying concentrations of the compounds. [b] Compared to DAMGO. [c] Compared to DPDPE. [d] Compared to U69,593. Data represent mean ± SEM.

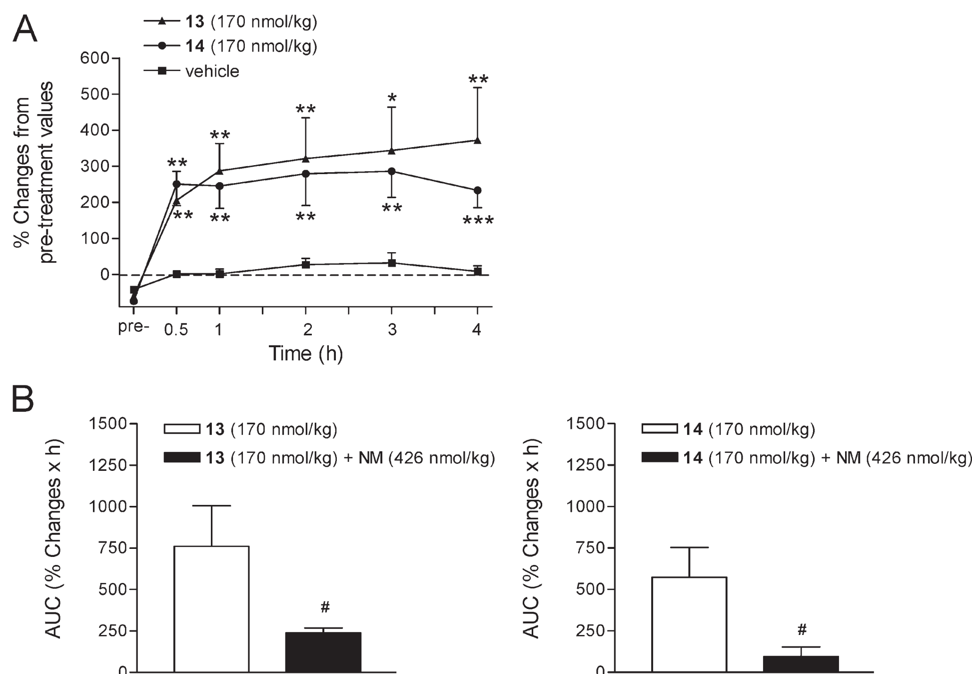


Figure 2. (A) Antinociceptive effects of 6-glycine substituted 14-phenylpropoxymorphinans **13** and **14** after sc administration to rats with carrageenan-induced inflammatory hyperalgesia. (B) Antagonism by naloxone methiodide (NM) on the antinociceptive effect of compound **13** and **14** after sc coadministration on the inflamed paw withdrawal latencies to mechanical stimulation. Values are presented as % changes in withdrawal latencies of the inflamed paw from the pretreatment (pre-) values obtained at 3 h after carrageenan injection. Areas under the curves (AUC) of the respective time curves are represented. Data are shown as mean ± SEM *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs vehicle-treated controls; #$p < 0.05$ vs agonist-treated animals.

include the clinically used buprenorphine,[49] nalfurafine (TRK-820) and its analogues,[50,51] and 14-aminomorphinone derivatives.[39,40] In the present study, the *N*-cyclopropylmethyl substituted compounds **17** and **18** and their corresponding *tert*-butyl esters **15** and **16** showed potent antinociceptive activities in rats after sc administration (Table 2). The in vivo pharmacology of derivatives **17** and **18** as antinociceptive agents was also supported by the functional data on the ligand-stimulated guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding to membranes from Chinese hamster ovary (CHO) stably transfected with human opioid receptors[21] (Table 3). Both compounds were highly potent partial agonists at the μ receptor and also at δ and κ receptors so that no significant selectivity for any receptor type was apparent. It is suggested that such compounds which interact with multiple receptor types may have an overall increased potency derived from the combined action at μ, δ, and κ opioid receptors.[52] Moreover, as found in this and our earlier studies[11,19-21] and also by others,[39,40,53-55] not necessarily the nature of the substituent at the nitrogen in morphine-like compounds but rather residues occupying a defined position in the vicinity to the morphinan nitrogen seem to be responsible for agonist/antagonist activity. Our SAR study also showed that replacement of the *N*-methyl group of compounds **13** and **14** with a cyclopropylmethyl group resulted in analogues **17** and **18**, respectively, which retained the high antinociceptive potency after sc administration (Table 2). This holds also true when evaluating the *N*-methyl substituted **3**[19] and naltrexone derivative **6**,[20] which show comparable antinociceptive potencies in different analgesic tests in mice after sc administration.

As shown in Table 2, esterification of the glycine residue in position 6 did not result in major alterations in antinociceptive activities and all *tert*-butyl esters were found to be greatly active displaying similar potencies to their corresponding glycine analogues. Notably, the *N*-cyclopropylmethyl *tert*-butyl ester **16** was about 6-fold more potent than derivative **18**. In the tail-flick test, antinociceptive potencies did not significantly differ between the α and β epimers, which is in agreement with the in vitro biological data where also no major changes in opioid binding affinities were observed. Their ED_{50} ratios were ranging between 0.6 and 2.4 after sc administration, except for the pair **15** vs **16**, where a 13-fold difference in the potency was calculated.

Opioids with hydrophilic groups such as amino acid residues attached to the C-6 position of the morphinan skeleton were targeted in an effort to obtain opioid compounds that would have potentially limited access to the CNS and thus to

reduce the activation of central opioid receptors. We have reported on the potent antinociceptive action of the 6-glycine conjugate of 14-*O*-methyloxymorphone, compound **10**, after systemic sc administration to rats with carrageenan-induced inflammatory hyperalgesia.[18] Further in vivo pharmacological studies were undertaken in the present study with the 14-phenylpropoxy derivative of **10**, compound **14**, and its corresponding 6α-glycine analogue **13** to assess their antinociceptive actions in a rat model of inflammatory pain. Subcutaneous administration of compounds **13** and **14**, in a dose of 170 nmol/kg, produced a significant attenuation in pain-related behavior in the inflamed paw by increasing withdrawal latencies to mechanical stimulation having a long duration of action (up to 4 h) (Figure 2A). These observations are in line with the earlier pharmacological findings on potent and prolonged inhibitory effects of the 14-methoxy 6-glycine analogue **10** in rats with inflammatory hyperalgesia.[18] Moreover, its antinociceptive actions after sc administration was shown to be mediated through peripheral mechanisms not only in carrageenan-induced hindpaw inflammation[18] but also in other rodent pain models including tail-flick test,[31] formalin test,[31] and acetic acid-induced writhing.[32] The antinociceptive efficacy of derivatives **13** and **14** in tested sc doses in carrageenan-induced inflammatory pain was comparable to that reported for compound **10** after a sc dose of 100 μg/kg (i.e., 183 nmol/kg) and with markedly longer duration of action than morphine and 14-methoxymetopon (**2**).[18] To evaluate whether the antinociceptive effect of the 14-phenylpropoxy substituted derivatives **13** and **14** in rats with carrageenan-induced hyperalgesia is peripherally mediated, naloxone methiodide, the peripherally acting opioid antagonist, was sc coadministered with each opioid. As shown in Figure 2B, naloxone methiodide (426 nmol/kg) significantly reversed the antinociceptive effect of a 170 nmol/kg dose of compound **13** and **14** on mechanical hyperalgesia. Hence, replacement of the 14-methoxy group in compound **10** with a phenylpropoxy group gave rise to new derivatives showing peripherally mediated antinociceptive action in a rat model of inflammatory pain. Outcomes of these studies extend our earlier reports on 6-amino acid substituted derivatives (i.e., glycine, alanine and phenylalanine) of 14-*O*-methyloxymorphone (**1**)[18,29−33] described as high affinity μ opioid receptor agonists that can induce potent antinociceptive effects via peripheral opioid receptor-specific mechanisms.

Conclusions

We have described the synthesis and biological activities of a series of new 6-glycine substituted 14-phenylpropoxymorphinans which emerged as high affinity and potent opioid antinociceptive agents. Specific modifications in the substitution pattern, such as introduction of a 14-phenylpropoxy group and a glycine residue at position 6, led to an interesting alteration in opioid activity by influencing the biological profile of these compounds interacting with μ, δ, and κ opioid receptors. On the basis of the SAR that has emerged, it could be observed that there is a significant enhancement in affinity at all opioid receptor types upon introduction of a phenylpropoxy group at position 14 in 6-glycine substituted morphinans, with most pronounced increases in activity at δ and κ receptors, leading to a loss in μ receptor selectivity. In vitro binding affinities to all opioid receptors and selectivity were not appreciably affected upon substitution of the *N*-methyl with a cyclopropylmethyl group. Besides the increased affinity for opioid receptors, another attribute of the new 6-glycine substituted 14-phenylpropoxymorphinans is the potent and long-lasting antinociceptive effects in rats after sc administration, displaying considerably increased potency than morphine. For the *N*-methyl derivatives **13** and **14**, antinociceptive potencies were in the range of their 14-methoxy analogues. Even derivatives **15**−**18** having a *N*-cyclopropylmethyl substituent acted as highly active antinociceptive agents, being several fold more potent than morphine. Subcutaneous administration of the *N*-methyl 6α- and 6β-glycine substituted 14-phenylpropoxymorphinans **13** and **14**, respectively, significantly reduced mechanical hypersensitivity in rats with carrageenan-induced inflammatory pain, effects shown to be mediated via activation of peripheral opioid receptors. Such peripherally acting opioids may represent novel drug candidates for pain treatment.

Experimental Section

General Methods. The starting material thebaine was obtained from Tasmanian Alkaloids Ltd., Westbury, Tasmania, Australia. Melting points were determined on a Kofler melting point microscope and are uncorrected. ^{1}H NMR spectra were obtained on a Varian Gemini 200 (200 MHz) spectrometer using tetramethylsilane (TMS) as internal standard for $CDCl_3$ and 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) for D_2O. Coupling constants (*J*) are given in Hz. IR spectra were taken on a Mattson Galaxy FTIR series 3000 in KBr pellets (in cm^{-1}). Mass spectra were recorded on a Varian MAT 44 S apparatus. Elemental analysis was performed at the Institute of Physical Chemistry of the University of Vienna, Austria. For column chromatography (MPLC), Silica Gel 60 (0.040−0.063 mm, Fluka, Switzerland) was used. Thin-layer chromatography (TLC) was performed on silica gel plates Polygram SIL G/UV254 (Macherey-Nagel, Germany) with CH_2Cl_2/MeOH/NH_4OH 90:9:1 as an eluent.

Radioligands [^{3}H]DAMGO, [^{3}H]U69,593, and [^{35}S]GTPγS were purchased from PerkinElmer (Boston, MA, USA). [^{3}H][Ile^{5,6}]deltorphin II was obtained from the Institute of Isotopes Co. Ltd. (Budapest, Hungary). Naloxone hydrochloride, tris-(hydroxymethyl)-aminomethane (Tris), carrageenan (λ-carrageenan), naloxone methiodide, GTPγS, guanosine diphosphate (GDP), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were obtained from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial sources. Compounds **11**, **12**, **15**, and **16** were used as bases and compounds **13**, **14**, **17**, and **18** were used as hydrochloride salts. Purities of tested compounds were determined by elemental analysis and were ≥95%.

General Procedure for the synthesis of 13, 14, 17, and 18 (13 as example). **11** (0.18 g; 0.35 mmol) was dissolved in 5 mL of 4 M HCl in dioxane and stirred under reflux for 2 h. The colorless crystals were filtered off under nitrogen, washed with anhydrous diethyl ether, and dried.

[[4,5α-Epoxy-3-hydroxy-17-methyl-14β-[(3-phenylpropyl)oxy]-morphinan-6α-yl]amino]acetic Acid Dihydrochloride (13·2HCl). Colorless crystals (89%); mp > 207 °C (dec). IR (KBr) 1744 (C=O). ^{1}H NMR (D_2O) δ 7.47−7.35 (m, 5 arom H), 6.96 (d, *J* = 8.3, 1 arom H), 6.87 (d, *J* = 8.3, 1 arom H), 5.09 (d, *J* = 3.2, *H*-C(5)), 3.01 (s, CH_3N). MS (ESI) *m*/*z* 479 [M + 1]$^+$. Anal. ($C_{28}H_{34}N_2O_5 \cdot 2HCl \cdot 1.9H_2O$) C, H, N.

[[4,5α-Epoxy-3-hydroxy-17-methyl-14β-[(3-phenylpropyl)oxy]-morphinan-6β-yl]amino]acetic Acid Dihydrochloride (14·2HCl). Colorless crystals (93%). mp > 217 °C (dec). IR (KBr) 1740 (C=O). ^{1}H NMR (D_2O) δ 7.44 (s, 5 arom H), 6.94 (s, 2 arom H), 4.96 (d, *J* = 7.0, *H*-C(5)), 2.99 (s, CH_3N). MS (ESI) *m*/*z* 479 [M + 1]$^+$. Anal. ($C_{28}H_{34}N_2O_5 \cdot 2HCl \cdot 2H_2O$) C, H, N.

[[17-Cyclopropylmethyl-4,5α-epoxy-3-hydroxy-14β-[(3-phenylpropyl)oxy]morphinan-6α-yl]amino]acetic Acid Dihydrochloride (17·2HCl). Colorless crystals (97%); mp > 218 °C (dec). IR (KBr) 1741 (C═O). ^{1}H NMR (D_2O) δ 7.29 (s, 5 arom H), 6.85 (d, J = 8.2, 1 arom H), 6.75 (d, J = 8.2, 1 arom H), 4.99 (d, J = 3.0, H-C(5)), 0.93 (m, CH-cp), 0.66 (m, CH_2-cp), 0.36 (m, CH_2-cp) (cp = cyclopropyl). MS (CI) m/z 519 [M + 1]$^+$. Anal. ($C_{31}H_{38}N_2O_5$·2HCl·1.4H_2O) C, H, N.

[[17-Cyclopropylmethyl-4,5α-epoxy-3-hydroxy-14β-[(3-phenylpropyl)oxy]morphinan-6β-yl]amino]acetic Acid Dihydrochloride (18·2HCl). Colorless crystals (89%); mp > 223 °C (dec).IR (KBr) 1741 (C═O).^{1}H NMR (D_2O) δ 7.42 (s, 5 arom H), 6.94 (s, 2 arom H), 4.99 (d, J = 7.0 H-C(5)), 1.02 (m, CH-cp), 0.82−0.68 (m, CH_2-cp), 0.46 (m, CH_2-cp) (cp = cyclopropyl.MS (CI) m/z 519 [M + 1]$^+$. Anal. ($C_{31}H_{38}N_2O_5$·2HCl·1.7H_2O) C, H, N.

Opioid Receptor Binding Assays. Membranes were prepared from Sprague−Dawley rat or guinea pig brains, obtained from the Institut für Labortierkunde and Laborgenetik, Medizinische Universität Wien (Himberg, Austria), as previously described.[30] Binding experiments were performed in 50 mM Tris-HCl buffer (pH 7.4.) in a final volume of 1 mL containing 0.3−0.5 mg of protein and at least 10 concentrations of test compound as described.[18] Rat brain homogenates were incubated either with [^{3}H]DAMGO (1 nM, 45 min, 35 °C) or [^{3}H][Ile^{5,6}]deltorphin II (0.5 nM, 45 min, 35 °C). Guinea pig brain homogenates were incubated with [^{3}H]U69,593 (1 nM, 30 min, 30 °C). Reactions were terminated by rapid filtration through Whatman glass fiber filters using a Brandel M24R cell harvester, followed by washing with 50 mM Tris-HCl buffer (pH 7.4). Nonspecific binding was determined using 10 μM naloxone. The bound radioactivity was measured by liquid scintillation counting. Inhibition constant (K_i) values were calculated from competition binding curves using the nonlinear least-squares curve fitting by GraphPad Prism program. All experiments were performed in duplicate and repeated at least three times.

[^{35}S]GTPγS Functional Assays. Functional assays were conducted on human opioid receptors stably transfected into CHO cells as described.[21] Aliquots of cell membranes (15 μg) in buffer A (20 mM HEPES, 10 mM $MgCl_2$ and 100 mM NaCl, pH 7.4) were incubated with 0.05 nM [^{35}S]GTPγS, 10 μM GDP, and test compounds, in a total volume of 1 mL, for 60 min at 25 °C. Nonspecific binding was determined using 10 μM GTPγS. Samples are filtered over glass fiber filters and counted as described for binding assays. Potency (EC_{50}, nM) and percentage of stimulation (% stim) with respect to the standard agonists DAMGO (μ), DPDPE (δ), and U69,593 (κ) were calculated using GraphPad Prism program. The results are mean ± SEM from at least three determinations, each performed in triplicates.

In Vivo Assays. General Methods. Male Wistar rats (120−150 g) used in the tail-flick test were purchased from Charles River (Budapest, Hungary). Male Sprague−Dawley rats (250 g) used in carrageenan-induced inflammatory pain were purchased form B&K Universal Lab (Sollentuna, Sweden). Animals were maintained on a 12 h light/dark cycle with free access to food and water at all times except during testing. Experiments were carried out in accordance to the Declaration of Helsinki and the Guide for Care and Use of Laboratory Animals. The experimental protocols were approved by the Ethical Board of Semmelweis University, Budapest, and Ethics Committee for Animal Research (North Stockholm, Sweden).

Tail-Flick Test. The tail-flick test was performed according to the described procedure.[31] A beam light was focused on the tip of the tail and the latency required for the rat to remove its tail was determined before (basal latencies) and after drug administration, using an arbitrary cutoff time of twice the basal reaction time. Compounds were dissolved in distilled water and administered sc to rats in a volume of 5 mL/kg of body weight. Each drug dose or vehicle (distilled water) was tested in at least five animals. The effective dose 50% (ED_{50}) values and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[56]

Carrageenan-Induced Hindpaw Inflammation. Unilateral inflammation was induced to nonanesthetized rats by injection of 100 μL of 1% carrageenan into the plantar surface of the right hindpaw as previously described.[18] All treatments were performed 3 h after carrageenan injection. Compounds were dissolved in physiological saline (0.9%) and administered sc in a volume of 100 μL. Control animals received the same volume of sc physiological saline solution. Each experimental group included four to seven animals. The Randall−Selitto test (Ugo Basile) was used to assess the hindpaw withdrawal latencies to mechanical stimulation as described.[18] A wedge-shaped, blunt piston with an area of 1 × 10 mm^2 and a loading rate of 48 g/s was applied to the dorsal surface of the manually handled hindpaw, and the time required to initiate the struggle response was assessed and expressed in seconds. A cutoff time of 20 s was applied. Withdrawal latencies to mechanical stimulation were measured before injection of carrageenan (basal values), prior to administration of the drug 3 h postcarrageenan (pretreatment values), and after drug administration. The antinociceptive response is expressed as percentage (%) changes in hindpaw withdrawal latencies from the pretreatment value obtained at 3 h after carrageenan injection, and was calculated as 100 $[(P_X - P_0)/P_0]$, where P_0 is the latency prior to drug administration at 3 h postcarrageenan and P_X is the latency after drug administration at corresponding time points.

Statistical Analysis. Data are expressed as mean ± SEM. The areas under the curves (AUC) were calculated by the trapezoidal rule using NCSS software. Statistical analysis was carried out using one-way analysis of variances (ANOVA) with Dunnett's post hoc test using SPSS software. A p value <0.05 was considered statistically significant.

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Supporting Information Available: Additional experimental, spectroscopic data, elemental analysis results, and supplementary pharmacology figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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